Surface analysis of biological materials using ToF-SIMS and laser post-ionisation

Nick Lockyer & *John Vickerman*

- Introduction
  - ToF-SIMS
  - Laser Post-Ionisation
- Analysis of biological materials
  - Challenges
  - Human Prostate Cancer Cells
  - Laser PI & the FEL
- Summary & Conclusions

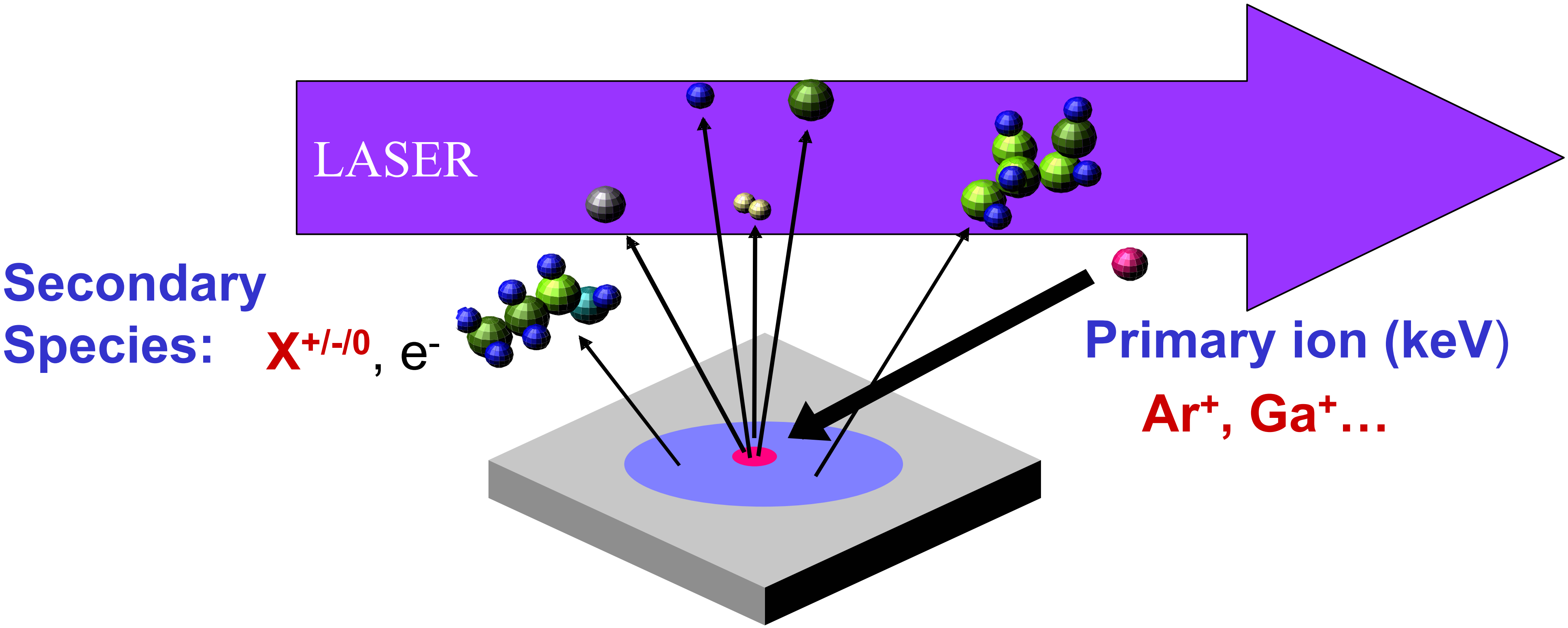
Surface Analysis Research Centre

UMIST

Secondary Ion Mass Spectrometry (SIMS)

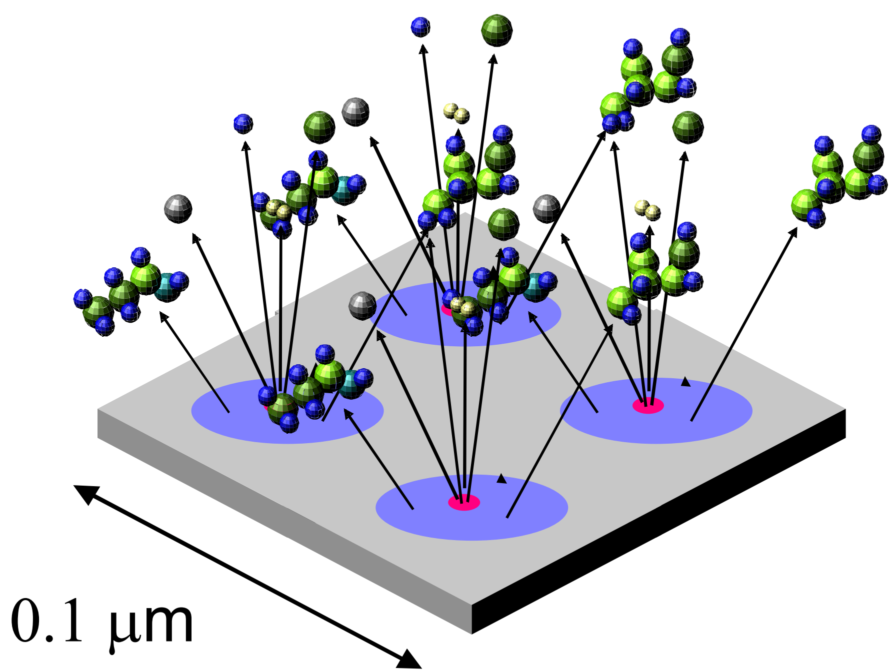
Laser Post-ionisation (Sputtered Neutral Mass Spectrometry)

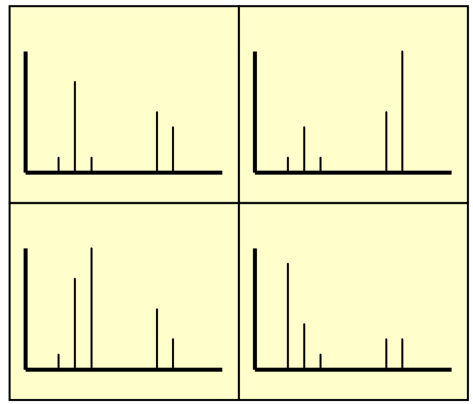
- *Primary* ion beam sputters atomic and molecular species from *top monolayer* of a solid surface
- 'Static SIMS' (<1% impacted) – detailed molecular characterisation
- *Secondary* ions are extracted and analysed by mass spectrometry



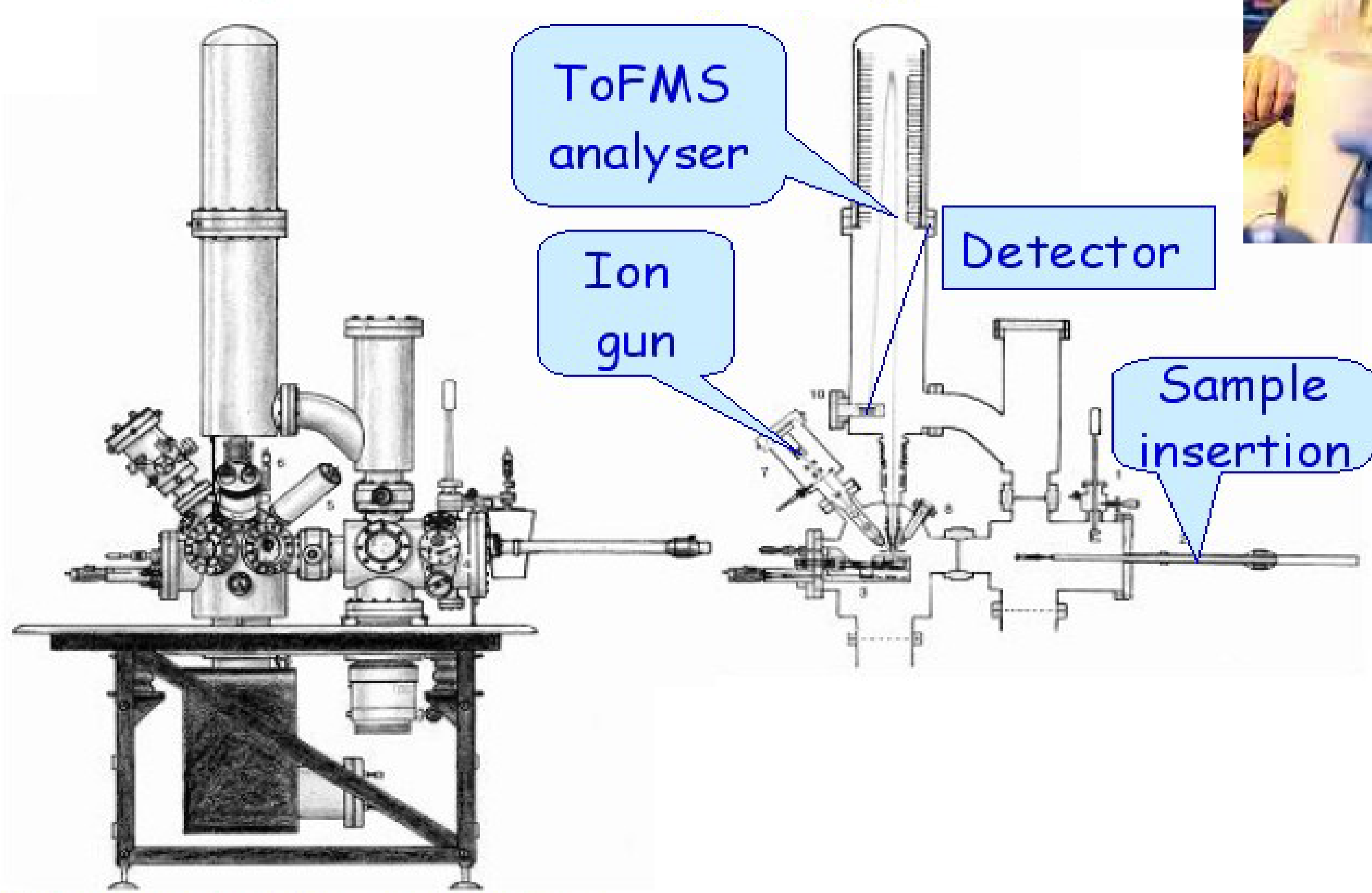
Retrospective Imaging - Chemical Microscopy

- Digitally scan focused ion beam over the surface
- FULL mass spectrum at each pixel.





ToF-SIMS - Instrumentation

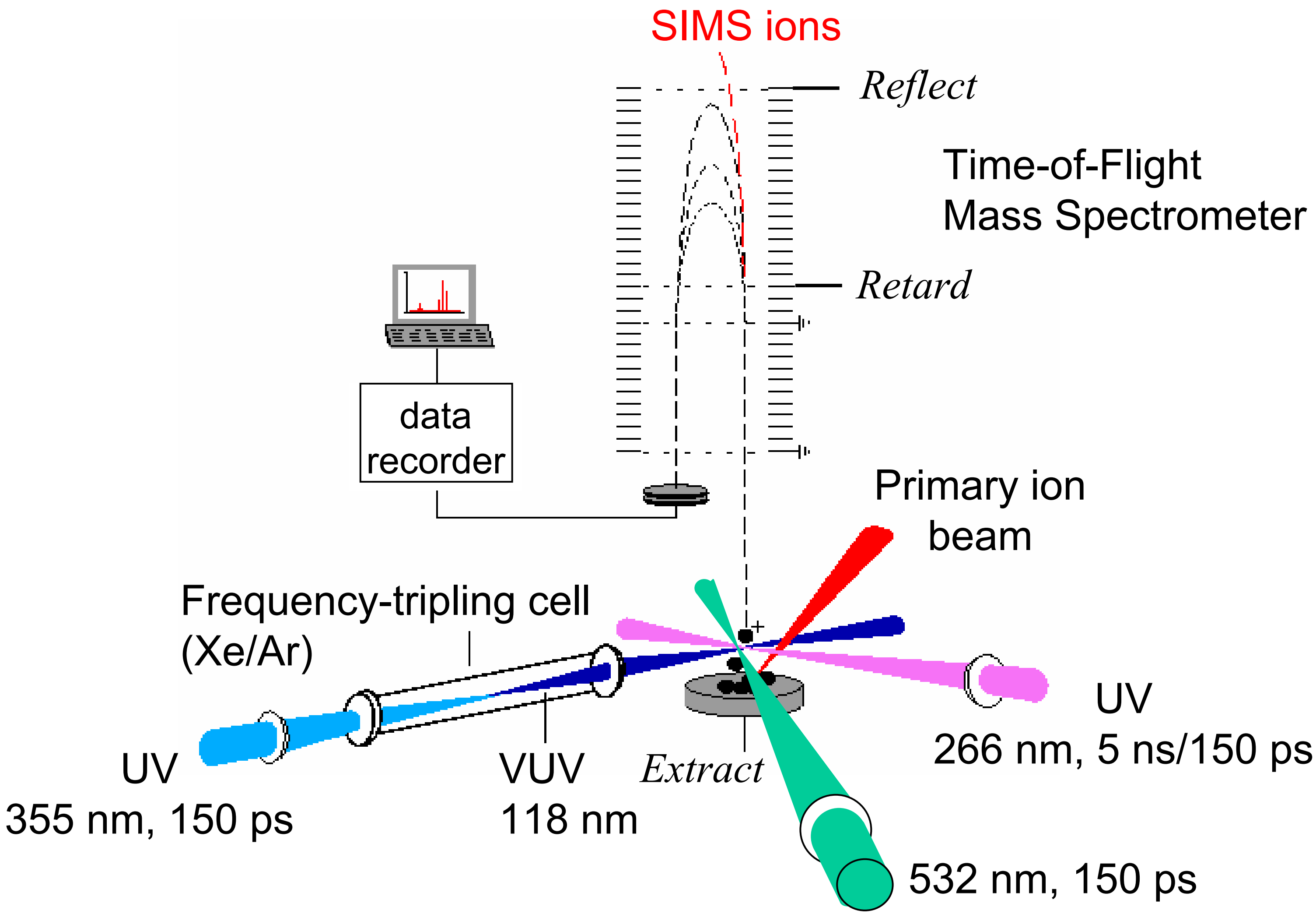


Laser Post-Ionisation - Mechanisms

- Single photon ionisation (SPI)
- Multiphoton ionisation (MPI)
  - resonant (REMPI)
  - non-resonant (NRMPI)

- Field Ionisation (FI)
  - high-intensity MPI
  - tunnel ionisation (TI)
  - barrier-suppression (BSI)

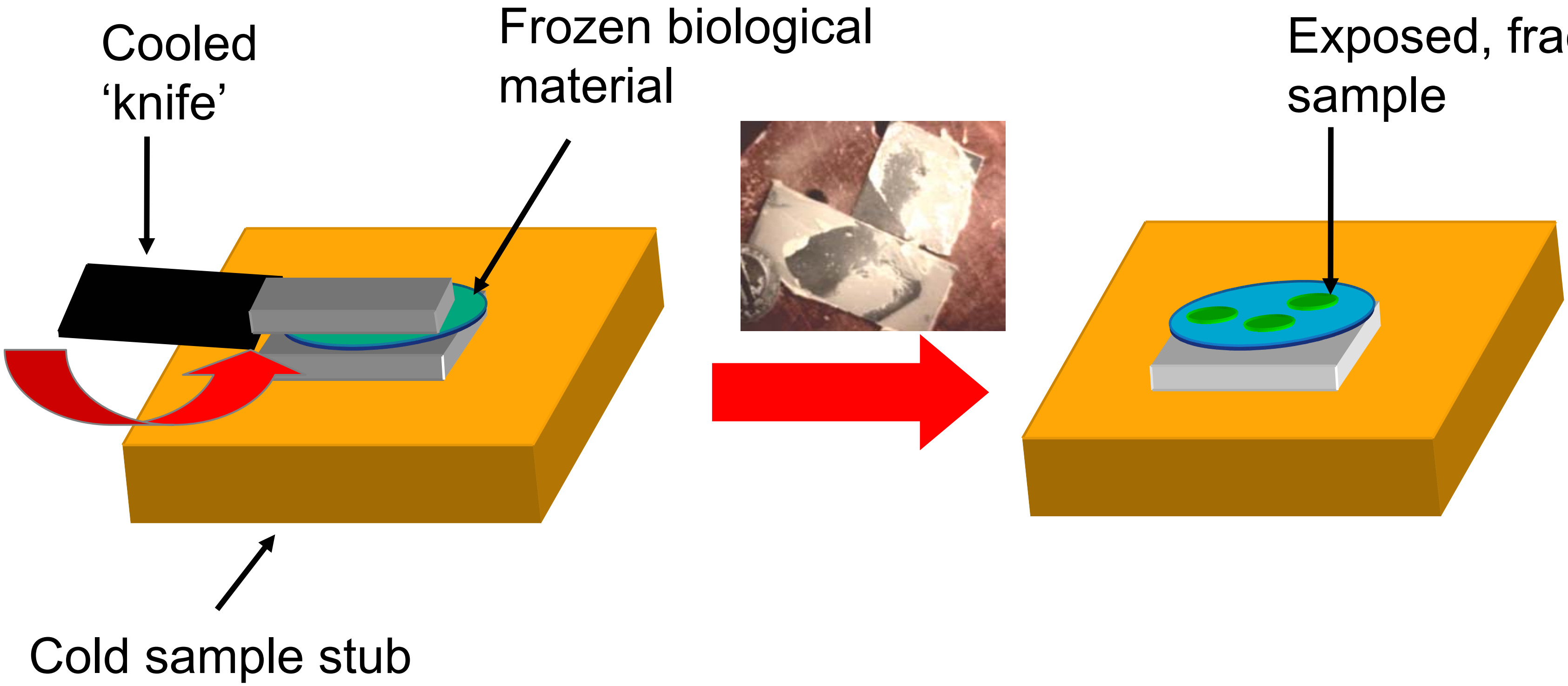
Laser Post-Ionisation



Bioanalysis – Challenges

- Sample preparation
  - Vacuum compatibility of biological cells
- Data interpretation
  - Complex mass spectra
  - Fragmentation
- Sensitivity
  - Low concentrations
  - Low secondary ion yields
  - Fragmentation

Sample Preparation – Fast Freezing & Freeze Fracture



- *in-situ* fracture (frozen hydrated surface for analysis)
- or *ex-situ* fracture & freeze dry

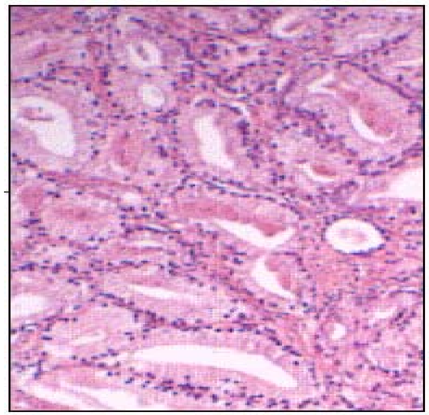
B. Cliff et al. *Rapid Commun. Mass Spectrom.* **17** (2003) 2163

ToF-SIMS Characterisation of Human Prostate Cancer cells

- Gleason *histopathological* Grading System

  - based on glandular architecture

Grade 2



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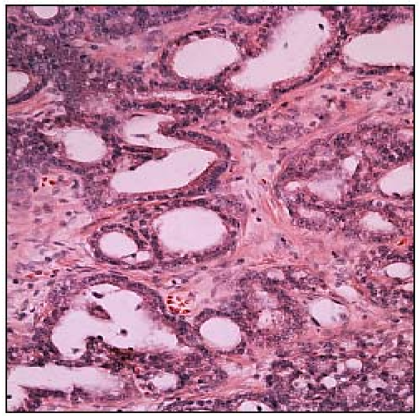

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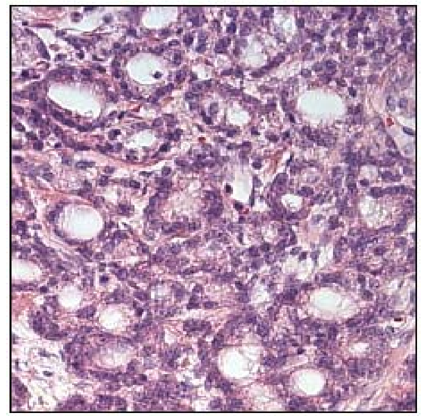
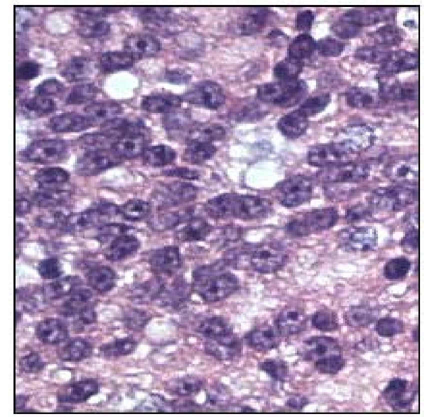


Grade 5



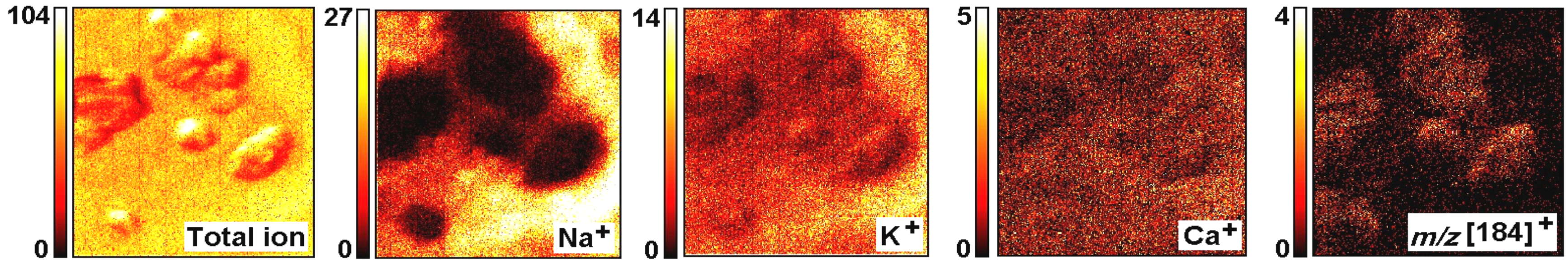
Disease progression

- Need a chemically based diagnostic tool to study basis of carcinogenesis

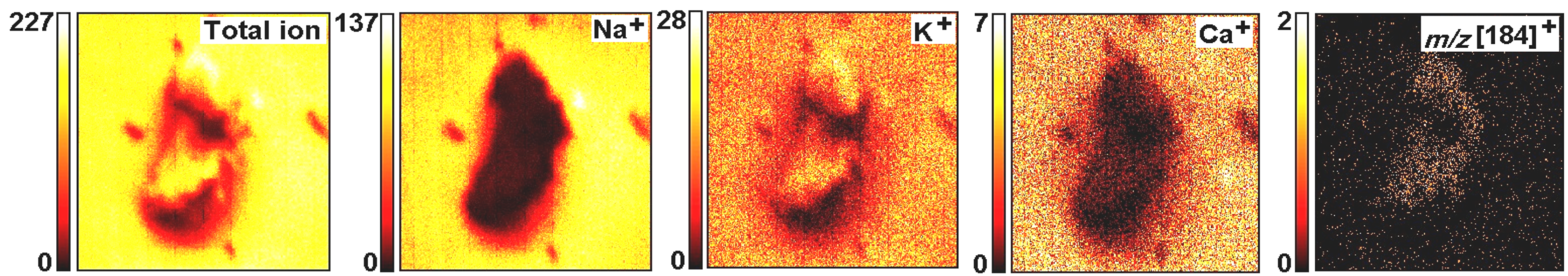
Data interpretation – Univariate Approach

- ToF-SIMS images of freeze-dried PC-3 cancer cells

Intact **150 μm f.o.v**



Fractured **100 μm f.o.v**

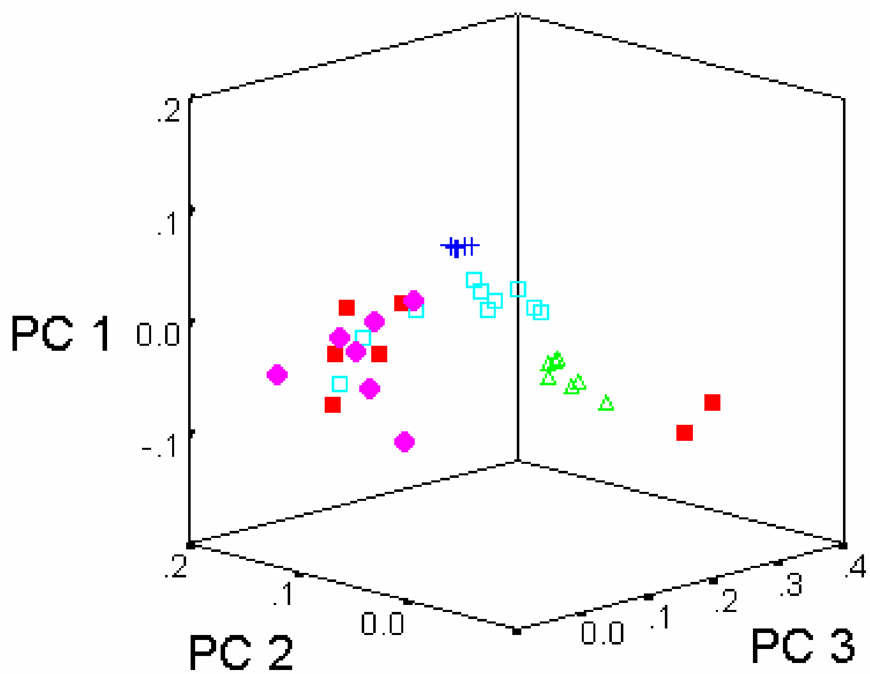


E.Gazi *et al.* Faraday Discussions 126 (in press)

Data interpretation – Multivariate Approach

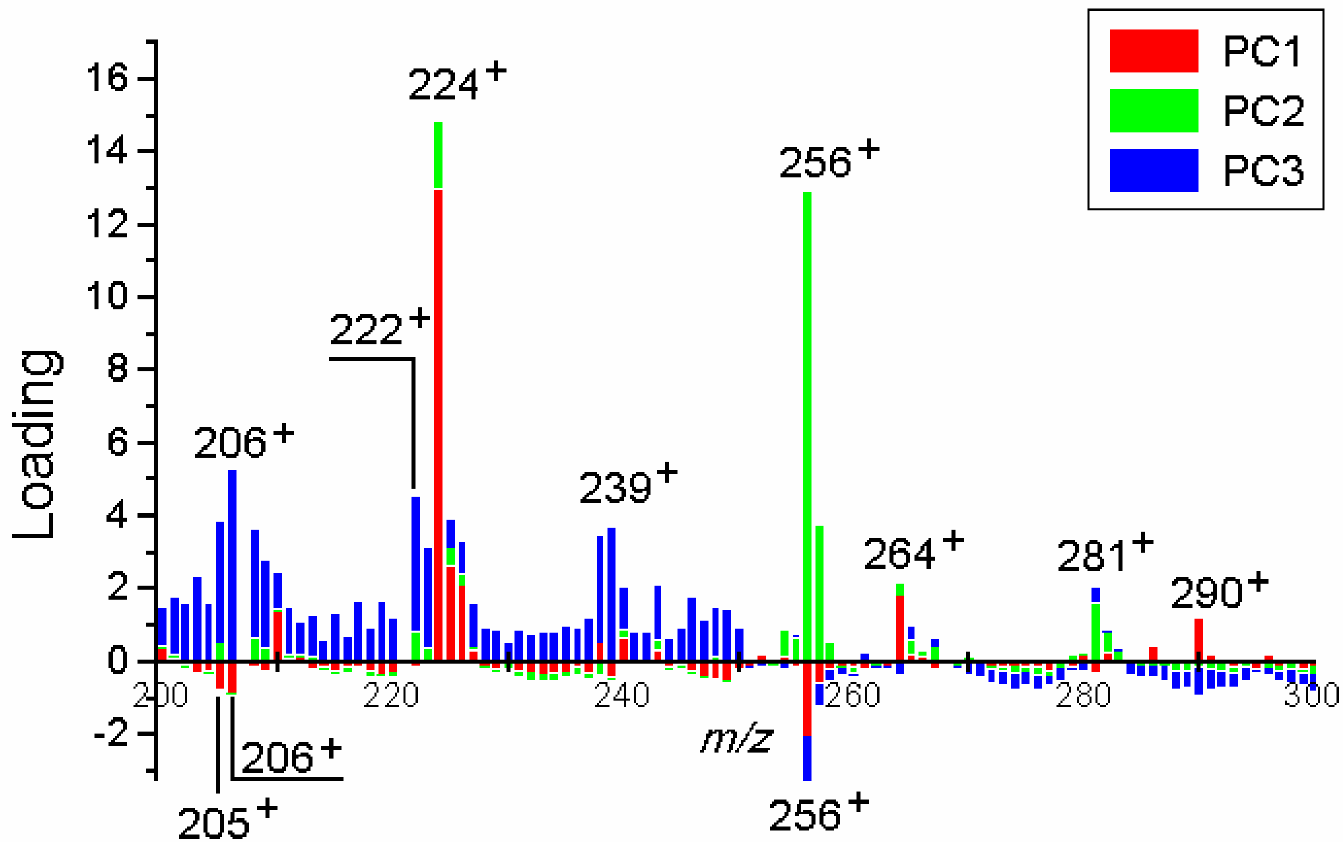
- Principal Component Analysis of cell lines

ToF-SIMS differentiates non malignant and malignant cancer cells derived from different metastatic sites



PNT2-C2: Transformed cells
EPC1/2: Epithelial cells
DU145: CaP (Brain metastasis)
PC-3: CaP (Bone metastasis)

Loadings plot for principal components 1-3 from CaP cell lines



- Helps interpret the scores plot
- Provides clues to the biochemistry underlying metastatic potential

Sensitivity – Molecular imaging

Pixel size	Pixel area	Molecules per pixel *	Atoms per pixel
10 μm x 10 μm	10^{-6} cm^2	4×10^8	2.5×10^9
1 μm x 1 μm	10^{-8} cm^2	4×10^6	2.5×10^7
500 nm x 500 nm	2.5×10^{-9} cm^2	1×10^6	6.25×10^6
100 nm x 100 nm	1×10^{-10} cm^2	40 000	2.5×10^5
200 Å x 200 Å	4×10^{-12} cm^2	1600	10 000

* assuming a molecular area of 5 Å x 5 Å

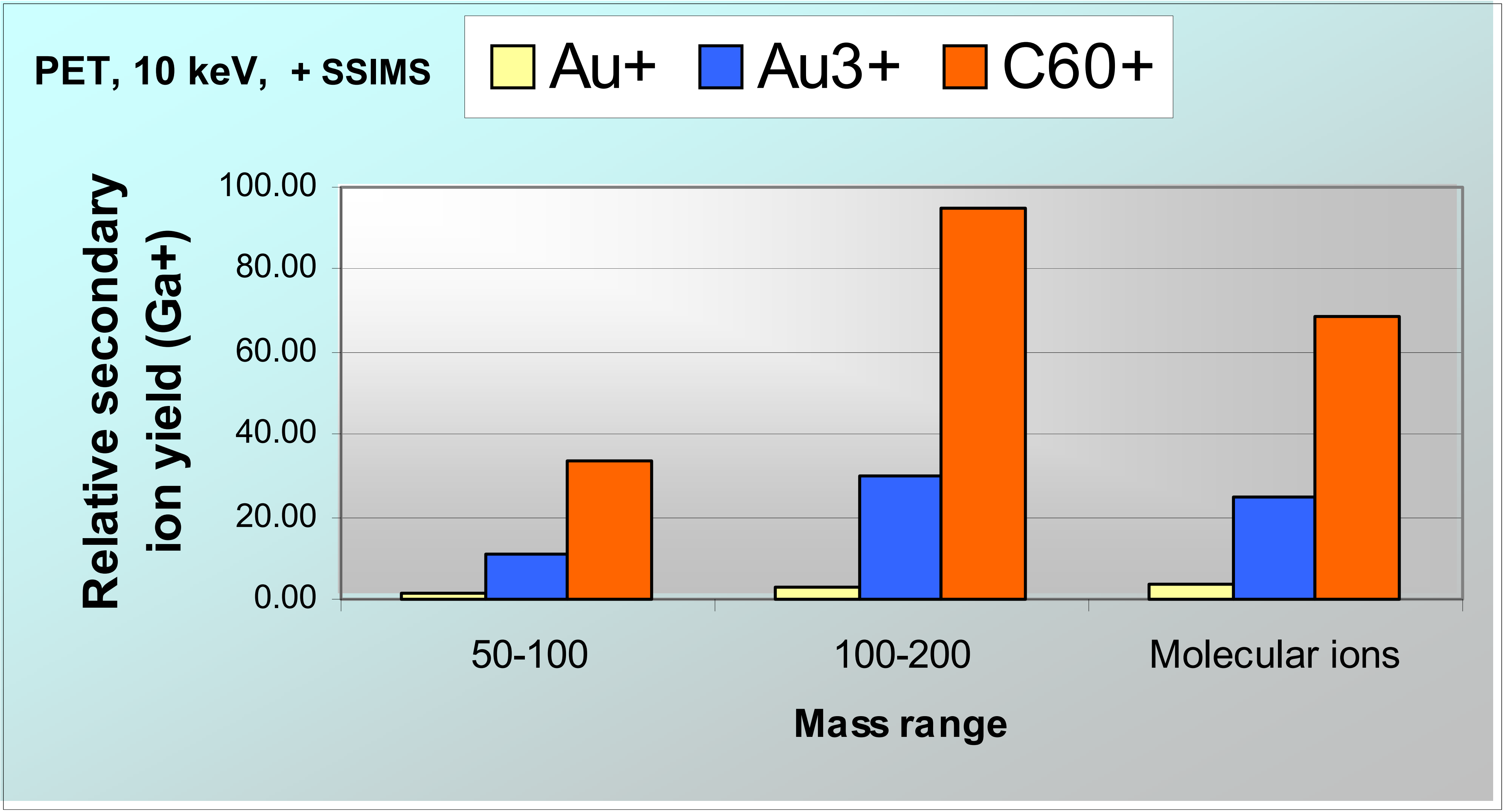
- *ToF-SIMS*
  - Static conditions (< 1% removed) => 10^4 molecules for analysis
  - Secondary ion yield ~10^{-5}-10^{-3} => ~ 0.1-10 ions/pixel

Sensitivity – increased ion yield

$$I_s^{+/-} = I_p Y_{chem} \alpha^{+/-} \theta_{chem} T$$

- Secondary ion yield of biomolecules (1k-10kDa) is very low < 10^{-5}
- 1990s: Polyatomics increase ions yields from sputtered organics
  - -Texas, large hydrocarbons, C_{60}^+
  - -Orsay, Au_n^+
  - -Munster,Idaho, SF_5^+
- 2001: UMIST/Ionoptika Ltd develop practical ToF-SIMS ion beam sources based on Au_n^+ and C_{60}^+

- Non-linear yield increases for polyatomics



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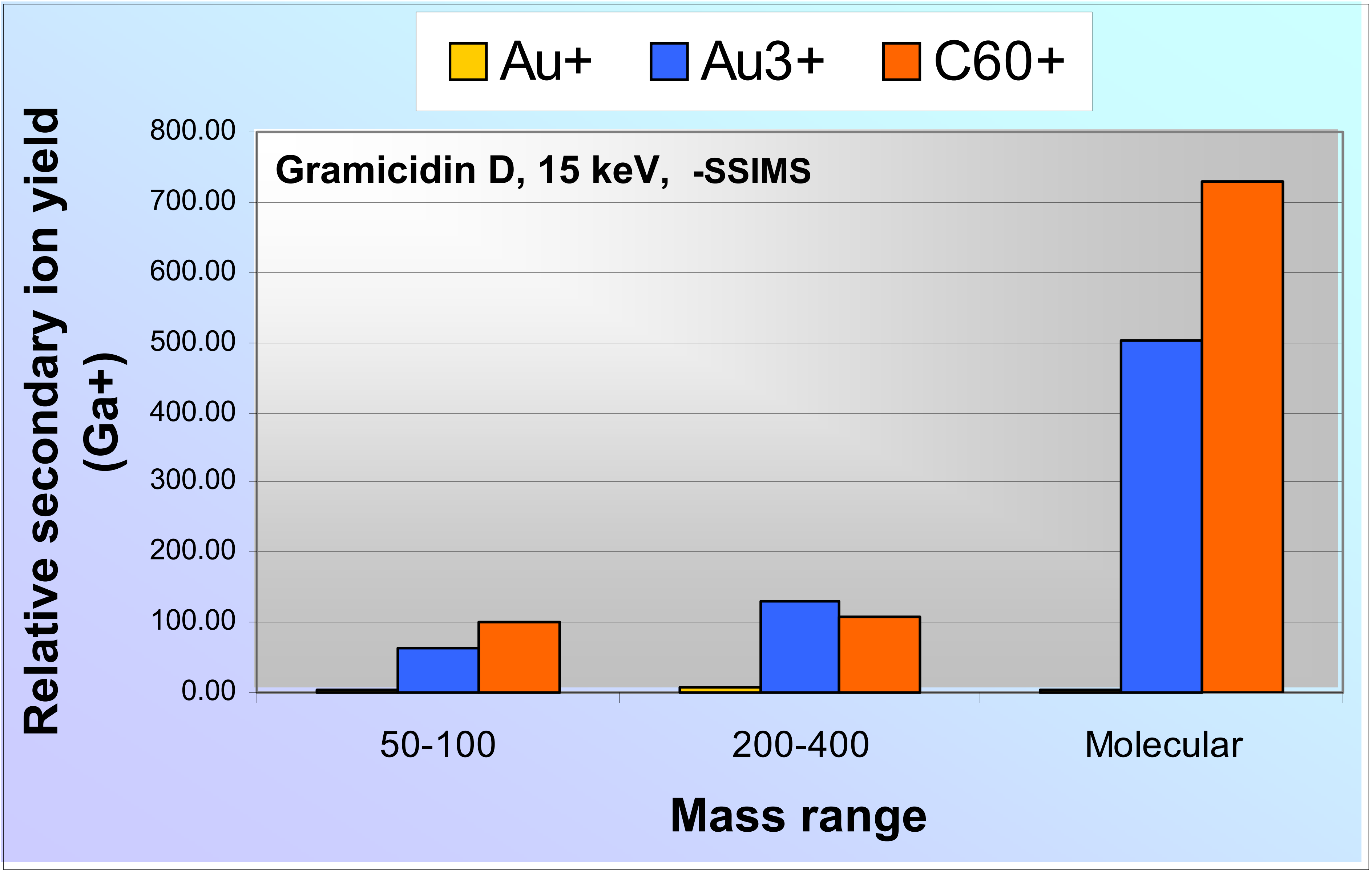
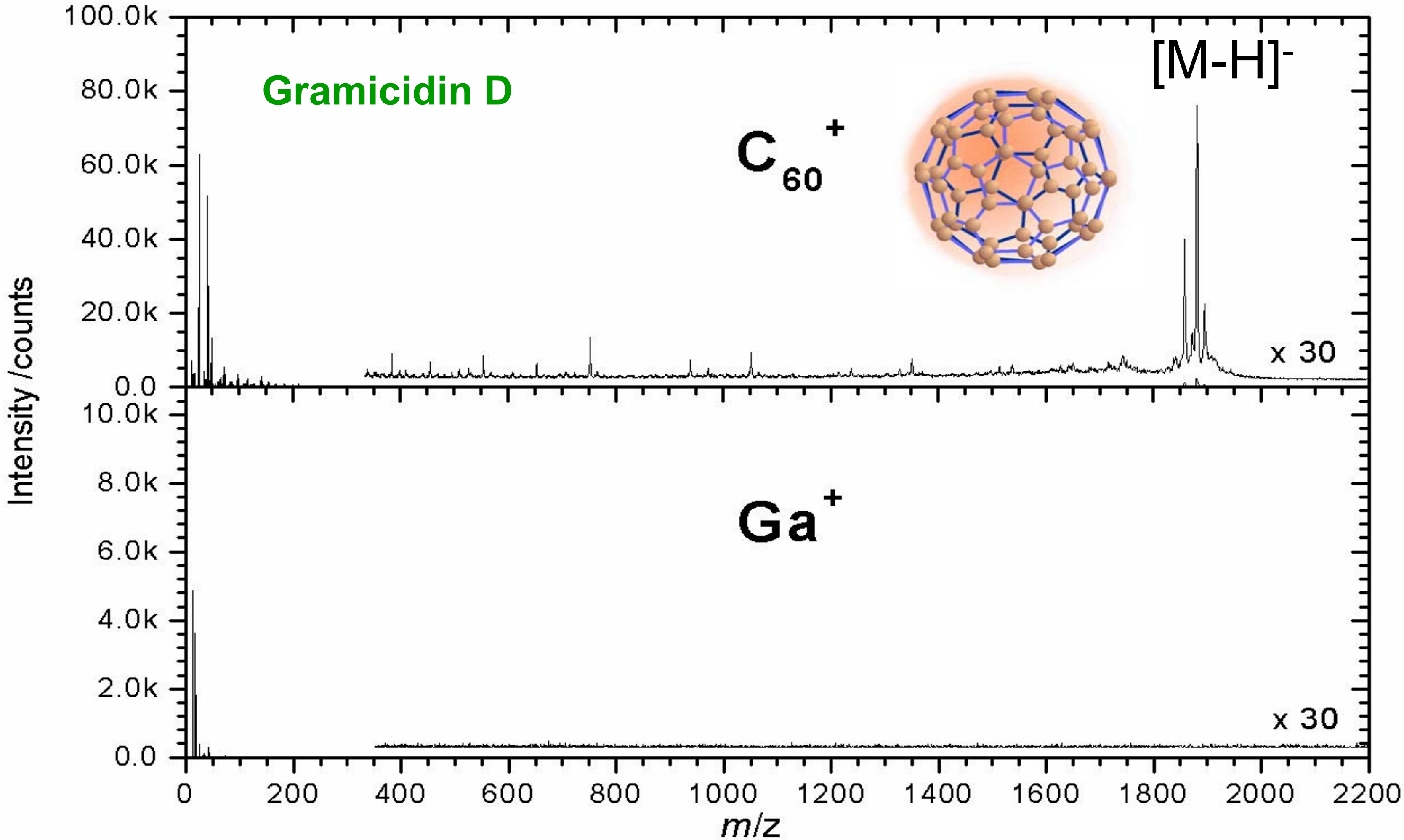


High-mass & polyatomic primary beams increase secondary ion yield



D. Weibel *et al. Anal. Chem.* 75 (2003) 1754

Mechanism of Secondary Ion Yield Enhancement with polyatomics?

- Impact energy spread over wider area.
- Impact energy dissipated very close to surface - greatest effect with C_{60}.
  - => Multiple sputtering events
  - => Non-linear enhancement

- What is the mechanism of emission?
- What is the effect on the internal energy of sputtered molecules?
- What about the neutral yield?

Threshold ionisation with tuneable VUV?

Sensitivity - Post-ionisation

$$I_s^0 = I_p Y_{chem} \alpha^0 \theta_{chem} T$$

- Neutral yields typically orders of magnitude higher than ion yields
- Post-ionisation efficiency determined by:
  - photoionisation efficiency

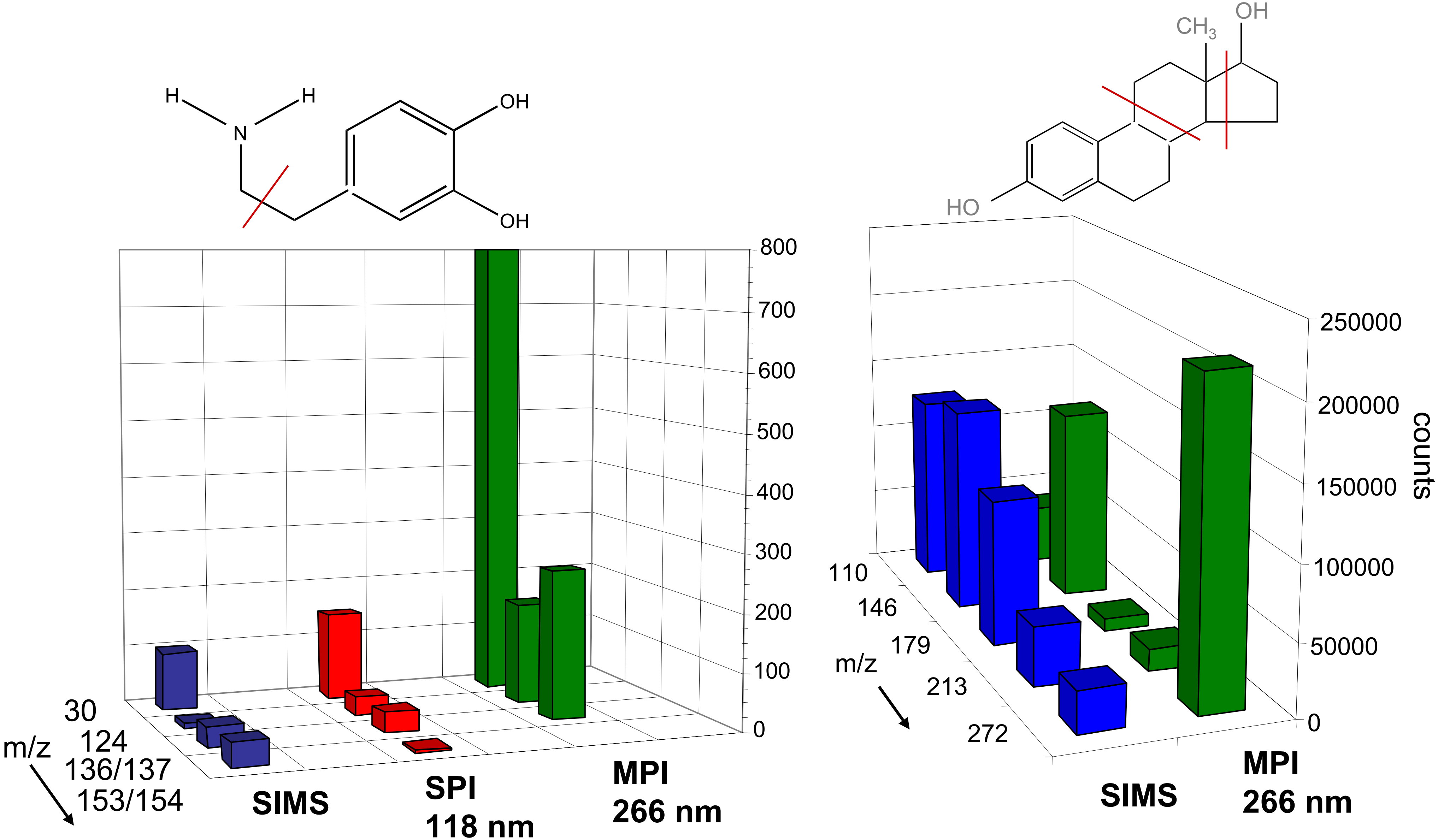
    $\leq$ 100% (atoms) $\leq$ 1-10% (organics) (SIMS $\alpha^{+/-} \leq 10^{-3}$)

    - Laser parameters: intensity, wavelength, pulse duration
    - Internal energy of desorbed species

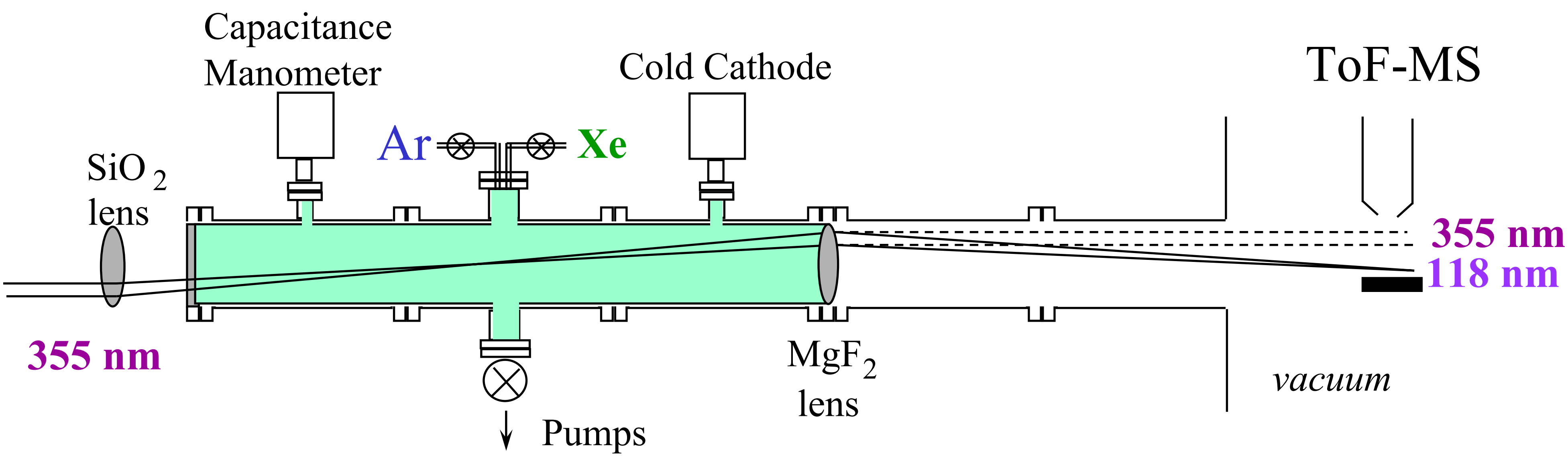
  - temporal/spatial overlap of post-ionisation volume with sputter plume

Increasing Sensitivity – Post-ionisation

- Modest increases for biomolecules under favourable conditions



Harmonic Generation of VUV photons for SPI



$$I(3\omega) \propto N^2 \, |\chi^{(3)}(\omega)|^2 \, I(\omega)^3 \, F(L,b,\Delta k)$$

- Conversion efficiency ~0.01%, 10^{12} photons/pulse at 118 nm

A.H. Kung *et al. Appl. Phys. Lett.* **22** (1973) 301 & **28** (1976) 239

U. Schühle *et al. J. Am. Chem. Soc.* **110** (1988) 2323 (1988)

Molecular post-ionisation - Fragmentation

Multiphoton

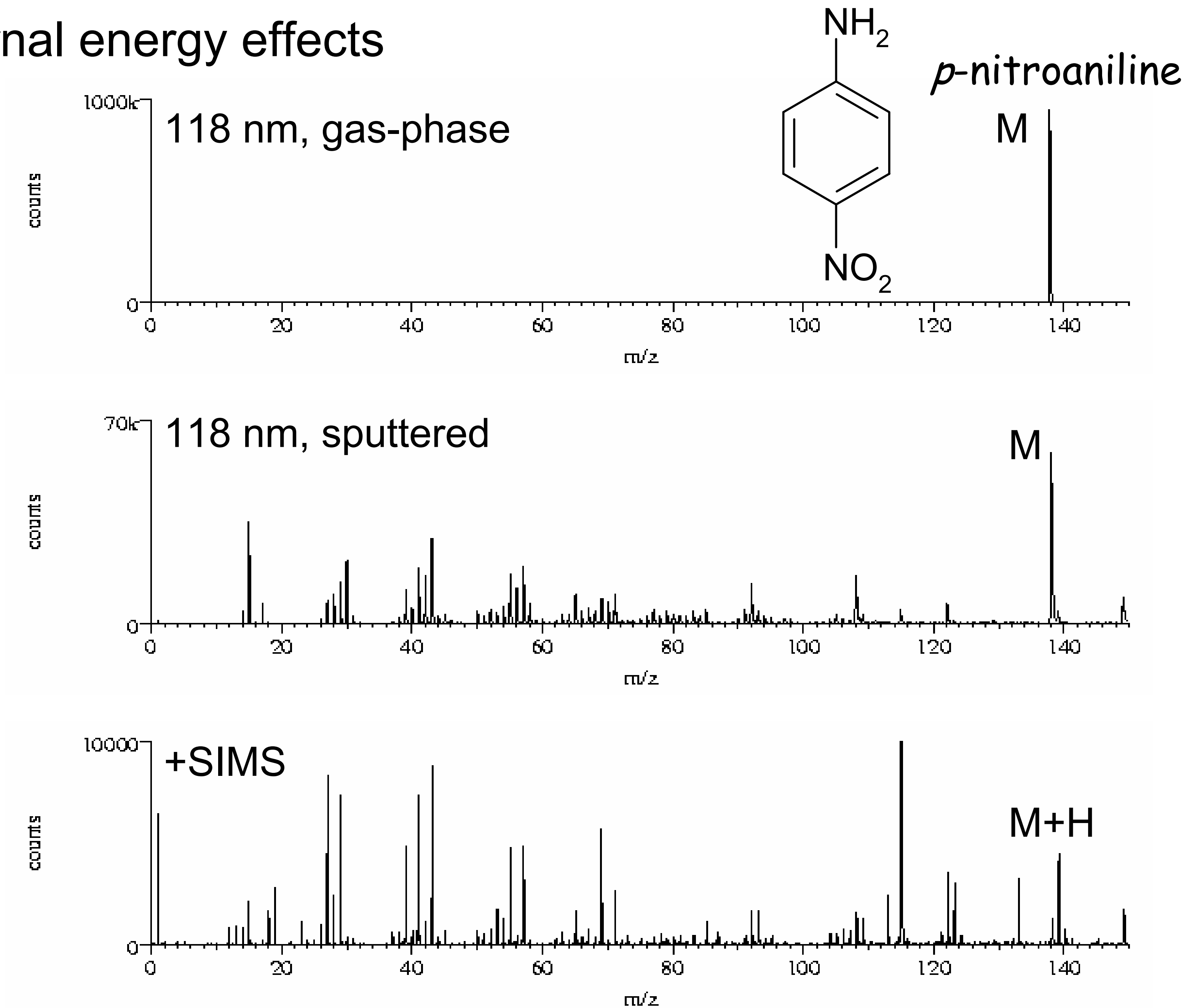
- Selectivity based on ‘resonance-enhancement’
  - Sputtered molecules occupy many vibrational modes
- Tuneable fragmentation
  - determined by laser parameters: I, λ, τ

Single photon

- Non selective ionisation
- ‘Soft’ ionisation
- Tuneable VUV (e.g. from the FEL)
  - Threshold ionisation (increased selectivity)
  - Tuneable fragmentation

Post-ionisation - Fragmentation

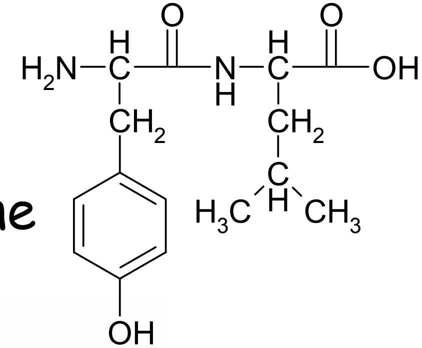
- Internal energy effects

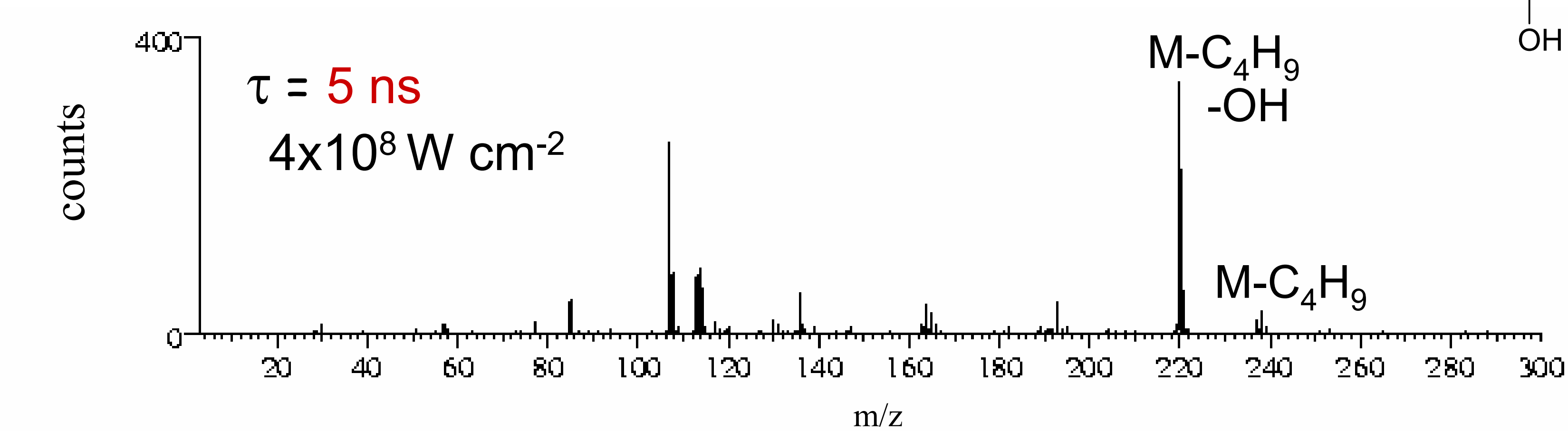


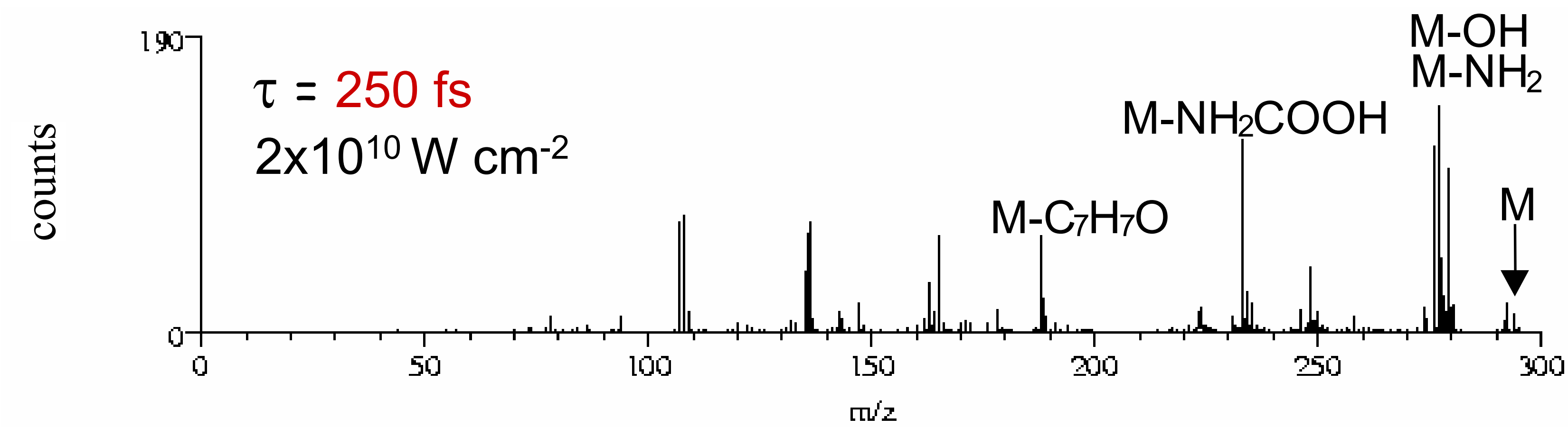
Post-ionisation - Fragmentation

- Ultrafast (ps-fs) pulses reduce MPI fragmentation

Tyrosyl-leucine







Summary - Biomolecular ToF-SIMS

- Cellular samples can be successfully prepared for ToF-SIMS/PI analysis using cryo-techniques

- Chemometric data analysis aids in the interpretation of complex data sets from biological samples

- Sensitivity is still a key issue in obtaining useful molecular information at the subcellular level
  - high-mass/polyatomics primary beams help
  - optimised post-ionisation would also help!!!

Future Directions

- Studies of fundamental cellular processes
- Providing information on the biochemical basis for disease progression at the cellular level
- Rapid identification of pathogenic microbes directly on food-stuffs, forensic samples etc.

Summary – Biomolecular Laser Post-ionisation

✓ Decoupled desorption & ionisation

– Independent optimisation of each step

✓ MPI - Some selectivity

- multiple chromophores, uncontrolled photon absorption
  $\Rightarrow$ fragmentation
- Ultrafast laser pulses reduce photofragmentation

✓ SPI - Non selective

- <1% ionisation efficiency, 10^{12} photon/pulse, 10^{5} W/cm^{2}

✓ Less complex mass spectra (?)

✓ Greater sensitivity (?)

– Sub femtomol detection limit

X Extra cost & complexity

Applications of Free Electron Laser in Biological SIMS/PI

- Spectroscopic investigation of sputtered ions and neutrals
  - Insight into mechanisms of sputtering regimes
- More control over ionisation and fragmentation of sputtered biomolecules
  - MPI with tuneable UV femtosecond pulses
  - SPI with tuneable VUV
- High sensitivity post-ionisation
  - $>10^{12}$ photons/pulse
  - 2-photon absorption may be a problem for SPI $\alpha^0>0.3$

Conclusion

Laser post-ionisation is a valuable complementary technique to ToF-SIMS offering many advantages in both applications and basic research.

Acknowledgements

ORGANIZING COMMITTEE FOR INVITATION

FUNDING:	**UK Engineering and Physical Sciences Research Council (EPSRC)**	
INSTRUMENT DEVELOPMENT:	**Prof. Nick Winograd, Penn State Univ.** **Kore Technology Ltd** **Ionoptika Ltd**	
POLYATOMICS:	**Dr. Daniel Weibel**	**Nathan Davies**
CELL WORK:	**Ehsan Gazi** **Dr. Harald Jungnickel** **Dr. Peter Gardner**	**Emrys Jones** **Dr. Ben Cliff** **Prof. John Dwyer**

Christies Hospital / Paterson Institute

Mr. Noel Clarke	**Dr. Mick Brown**
Dr. Jonathan Shanks	**Clare Hart**